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(54) **Isometric primer extension method and kit for detection and quantification of specific nucleic acid**

(57) A method for detecting and/or quantifying a target DNA or RNA present in a sample by a isometric primer extension method is disclosed. The method includes carrying out a primer extension reaction in the absence of a free nucleotide so that the primer extension reaction is stopped where the absent nucleotide would have been inserted. Thus, as the amount of incorporation of a labeled nucleotide on the primer extended product is detected, the amount of the target RNA or DNA in the sample is measured.

Fig. 1

## Target DNA Detection and Quantification

(1) Extension with dTTP, dGTP, dCTP, either with ddATP or without dATP

(SEQ ID NO:2)  
5' TGATGAGGCGTGMANTCGTCGTGCTGACGACGCCGCGATTCTGCTCTTATGGCGATAGCAT 3'  
3' gtcctcgcg Primer 2 3' cgttcgtcggtcgt (SEQ ID NO:3) Primer 1

(2) Extension with dATP, dGTP, dCTP, either with ddTTP or without dTTP

(3) Extension with dATP, dGTP, dTTP, either with ddCTP or without dCTP

Line represents primer sequence. Small letters a, g, c, and t are bases extended from the primer, some of which are labeled with a detectable marker. u and t are used interchangeably.



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# EUROPEAN SEARCH REPORT

Application Number  
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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	WO 96 30545 A (APPLIED GENETICS INC) 3 October 1996 (1996-10-03) * page 16, line 24-28 *	1-7, 9, 11-40	C12Q1/68
Y	* page 21, line 27-31 *	8	
X	PREZANT T R ET AL: "TRAPPED-OLIGONUCLEOTIDE NUCLEOTIDE INCORPORATION (TONI) ASSAY, A SIMPLE METHOD FOR SCREENING POINT MUTATIONS" HUMAN MUTATION, WILEY-LISS, NEW YORK, NY, US, vol. 1, no. 2, 1992, pages 159-164, XP000571693 ISSN: 1059-7794 * the whole document *	1-7, 9, 11-40	
Y		8	
X	US 5 846 710 A (BAJAJ S PAUL) 8 December 1998 (1998-12-08) * column 4, paragraph 4; claim 1 *	1-4, 7, 9	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
A	KORNHER J S ET AL: "MUTATION DETECTION USING NUCLEOTIDE ANALOGS THAT ALTER ELECTROPHORETIC MOBILITY" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 17, no. 19, 11 October 1989 (1989-10-11), pages 7779-7784, XP002047718 ISSN: 0305-1048 * the whole document *	1-9, 11-40	C12Q
		-/--	
The present search report has been drawn up for all claims			
Place of search <b>MUNICH</b>		Date of completion of the search <b>4 February 2004</b>	Examiner <b>Knudsen, H</b>
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.CI.7)
A	FAHY E ET AL: "Multiplex fluorescence-based primer extension method for quantitative mutation analysis of mitochondrial DNA and its diagnostic application for Alzheimer's disease" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 25, no. 15, 1997, pages 3102-3109, XP002133461 ISSN: 0305-1048 * the whole document *	1-9, 11-40	
Y	US 5 888 819 A (GOELET PHILIP ET AL) 30 March 1999 (1999-03-30) * the whole document *	8	
A		1-7, 9, 11-40	
P, X	EP 1 101 824 A (MORISAWA SHINKATSU ; WANG XIAO BING (US)) 23 May 2001 (2001-05-23) * claims 1-36; examples 1-3 *	1-9, 11-40	
X	BRAUN A ET AL: "Improved Analysis of Microsatellites Using Mass Spectrometry" GENOMICS, ACADEMIC PRESS, SAN DIEGO, US, vol. 46, no. 1, 15 November 1997 (1997-11-15), pages 18-23, XP004459084 ISSN: 0888-7543 * the whole document *	1-6, 10-13, 15, 18, 20-24, 34, 38	TECHNICAL FIELDS SEARCHED (Int.CI.7)
X	MUELLER SABINE ET AL: "Retention of imprinting of the human apoptosis-related gene TSSC3 in human brain tumors" HUMAN MOLECULAR GENETICS, vol. 9, no. 5, 22 March 2000 (2000-03-22), pages 757-763, XP002262092 ISSN: 0964-6906 * page 762 *	1-6, 10-13, 15, 18, 20-24, 34, 38	
		-/-	
The present search report has been drawn up for all claims			
Place of search <b>MUNICH</b>		Date of completion of the search <b>4 February 2004</b>	Examiner <b>Knudsen, H</b>
<p><b>CATEGORY OF CITED DOCUMENTS</b></p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons &amp; : member of the same patent family, corresponding document</p>			

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Application Number  
EP 01 30 4958

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (InCL.7)
A	US 5 849 542 A (SCHWARZ TEREK ET AL) 15 December 1998 (1998-12-15) * abstract * * column 3, line 49-67 * ----	1-6, 10-40	
A	WO 99 57318 A (HILLENKAMP FRANZ ; KOESTER HUBERT (US); SEQUENOM INC (US)) 11 November 1999 (1999-11-11) * page 78-84; claims 92-100, 138-145 * ----	31-38	
A	US 5 830 655 A (BECKER CHRISTOPHER HANK ET AL) 3 November 1998 (1998-11-03) * examples 6,8 * -----	31-38	
The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (InCL.7)
Place of search <b>MUNICH</b>		Date of completion of the search <b>4 February 2004</b>	Examiner <b>Knudsen, H</b>
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#### CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

#### LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

☒ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

☐ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:



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**LACK OF UNITY OF INVENTION  
SHEET B**

Application Number

EP 01 30 4958

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

**1. Claims: 1-6,11-40 (partly), 7-9 (completely)**

This invention concerns a primer extension method using one or two or three types of non-terminator nucleotide with or without a terminator nucleotide that is different from the non-terminator nucleotides wherein either the non-terminator nucleotide or the terminator nucleotide is labelled and the amount of labelling signal is detected or quantified on the primer extended nucleotides.

**2. Claims: 1-6,11-40 (partly), 10 (completely)**

This invention concerns a primer extension method using one or two or three types of non-terminator nucleotide with or without a terminator nucleotide that is different from the non-terminator nucleotides wherein the amount of extended primers is detected by mass spectrometry.

**ANNEX TO THE EUROPEAN SEARCH REPORT  
ON EUROPEAN PATENT APPLICATION NO.**

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This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on  
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04-02-2004

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9630545	A	03-10-1996	AU 5296496 A WO 9630545 A1 US 5976798 A	16-10-1996 03-10-1996 02-11-1999
US 5846710	A	08-12-1998	NONE	
US 5888819	A	30-03-1999	AU 660173 B2 AU 1584892 A CA 2105060 A1 EP 0576558 A1 FI 933870 A FI 20010223 A JP 6505394 T NO 933136 A WO 9215712 A1 US 2003044778 A1 US 2003044779 A1 US 5762876 A US 6004744 A	15-06-1995 06-10-1992 06-09-1992 05-01-1994 03-09-1993 07-02-2001 23-06-1994 02-09-1993 17-09-1992 06-03-2003 06-03-2003 09-06-1998 21-12-1999
EP 1101824	A	23-05-2001	CA 2319349 A1 CN 1297058 A EP 1101824 A2 JP 2001245698 A RU 2200762 C2	22-05-2001 30-05-2001 23-05-2001 11-09-2001 20-03-2003
US 5849542	A	15-12-1998	DE 69426731 D1 DE 69426731 T2 EP 0765401 A1 WO 9514108 A1 JP 9505397 T	29-03-2001 28-06-2001 02-04-1997 26-05-1995 27-05-1997
WO 9957318	A	11-11-1999	US 2001055811 A1 AU 3896799 A CA 2328110 A1 DE 19983215 T0 EP 1075545 A2 JP 2002513917 T NO 20005539 A WO 9957318 A2 US 6558902 B1 US 2003148528 A1	27-12-2001 23-11-1999 11-11-1999 17-05-2001 14-02-2001 14-05-2002 05-01-2001 11-11-1999 06-05-2003 07-08-2003
US 5830655	A	03-11-1998	US 5700642 A AU 695705 B2 AU 5635296 A	23-12-1997 20-08-1998 11-12-1996

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The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

04-02-2004

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5830655      A	CA	2220418 A1	28-11-1996
	CN	1191575 A	26-08-1998
	DE	69605669 D1	20-01-2000
	DE	69605669 T2	27-07-2000
	EP	0828855 A1	18-03-1998
	JP	3437184 B2	18-08-2003
	JP	11505127 T	18-05-1999
	WO	9637630 A1	28-11-1996

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